

9/530746
422 Rec'd PCT/PTO 04 MAY 2000

claims

1. Method for the detection of a nucleic acid comprising the steps

- producing a plurality of amplificates of a section of this nucleic acid with the aid of two primers, one of which can bind to a binding sequence (A) of one strand of the nucleic acid and the other can bind to a binding sequence C' which is essentially complementary to a sequence C which is located in the 3' direction from A and does not overlap A,
- contacting the amplificates with a probe having a binding sequence D which can bind to a sequence B located between the sequences A and C or to the complement thereof, and
- detecting the formation of a hybrid of the amplificate and probe,

wherein the sequence located between the binding sequences A and C contains no nucleotides or less than 3 nucleotides that do not belong to the sequence region E formed from the binding sequence D of the probe and the sequence of the amplificate bound thereto and the amplificates are shorter than 100 nucleotides.

2. Method as claimed in claim 1, wherein the binding sequence D of the probe overlaps one or both binding sequences of the primers.

3. Method as claimed in one of the previous claims, wherein at least one of the primers has nucleotides in its non-extendible part which do not hybridize directly with the nucleic acid to be detected or with its complement.
4. Method as claimed in one of the previous claims, wherein at least one of the binding sequences is not specific for the nucleic acid to be detected.
5. Method as claimed in one of the previous claims, wherein the total length of the amplicon does not exceed 74 nucleotides.
6. Method as claimed in one of the previous claims, wherein at least one of the primers is immobilizably-labelled and the probe is detectably-labelled.
7. Method as claimed in one of the previous claims, wherein at least one of the primers is detectably-labelled and the probe is immobilizably-labelled or is immobilized.
8. Method as claimed in one of the previous claims, wherein the probe is labelled with a fluorescence quencher as well as with a fluorescent dye.
9. Method as claimed in one of the previous claims, wherein one of the primers is labelled with a first energy transfer component and the probe is labelled with a second energy transfer component which is different from the first energy transfer component.

10. Method as claimed in one of the previous claims, wherein the amplificate is detected by physical and/or spectroscopic methods.
11. Method as claimed in one of the previous claims, wherein at least one of the primers is not specific for the nucleic acid to be detected.
12. Method as claimed in claim 11, wherein two of the primers are not specific for the nucleic acid to be detected.
13. Method as claimed in one of the claims 11 and 12, wherein the probe is not specific for the nucleic acid to be detected.
14. Method as claimed in one of the previous claims, wherein nucleotides which are each complementary to A, G, C and T are used in the amplification.
15. Method as claimed in one of the previous claims, wherein the amplificates are detected by means of mass spectroscopy.
16. Method for the specific detection of a nucleic acid comprising the steps
 - producing a plurality of amplificates of a section of this nucleic acid with the aid of at least two primers
 - contacting the amplificates with a probe which can bind to the amplificate and

- detecting the formation of a hybrid of the amplificate and the probe,

wherein at least one of the primers is not specific for the group of organisms to which the organism to be detected belongs and the amplificates are shorter than 100 nucleotides.

17. Method as claimed in claim 16, wherein two of the primers are not specific for the nucleic acid to be detected.
18. Method as claimed in one of the claims 16 and 17, wherein the probe is not specific for the nucleic acid to be detected.
19. Method as claimed in one of the claims 16 to 18, wherein nucleotides which are each complementary to A, G, C and T are used in the amplification.
20. Method for the simultaneous production of amplificates of parts of nucleic acids in which primers are used which allow an amplification of these parts having different sequences, wherein the primers are selected such that the amplificates that are formed do not differ by more than 20 % in length and are not longer than 100 nucleotides.
21. Method as claimed in claim 20, wherein amplificates and nucleic acids of HIV, HBV and HCV are produced simultaneously.

22. Method for the detection of HCV, wherein two primers and one probe are used whose sequences are derived from sequences of consecutive bases of the HGBV sequence shown below, complementary sequences thereto or sequences that are more than 80 % identical to these sequences:

5'-GTACTGCCTG ATAGGGTCCT TGCGAGGGGA TCTGGGAGTC
TCGTAGACCG TAGCACATG-3'

23. Method as claimed in claim 22, wherein HCV-RNA is detected.

24. Method for the detection of several nucleic acids comprising the steps

- simultaneous production of a plurality of amplificates of sections of these nucleic acids with the aid of pairs of two primers, of which in each case one can bind to a binding sequence (A) of a strand of the nucleic acid and of which the other can bind to a binding sequence C' which is essentially complementary to a sequence C which is located in the 3' direction from A and does not overlap A, wherein the primers are selected such that the amplificates that are formed do not differ by more than 20 % in length and are not longer than 100 nucleotides,
- contacting the respective amplificates with a probe each time having a binding sequence D which can bind to the sequence B located between the sequences A and C or to the complement thereof, and

- detecting the formation of a hybrid of the amplificate and a probe,

wherein the sequence located between the binding sequences A and C contains no nucleotides or less than 3 nucleotides that do not belong to the sequence section E formed from the binding sequence D of the probe and the sequence of the amplificate bound thereto and the amplificates are shorter than 100 nucleotides.

25. Method as claimed in claim 24, wherein amplificates of nucleic acids of HIV, HBV and HCV are produced simultaneously.